212-Plat
Molecular Dynamics Simulations of Ribosomes: Integrating Theory and Experiment
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We will report results from the first molecular simulations of eukaryotic ribosomes. Using an integrated approach, we combine data from X-ray crystallography, cryo-EM and SHAPE chemical probing. Over the past decade, we have developed a pipeline that begins with X-ray crystallographic structures and uses molecular simulation to produce all-atom models consistent with cryo-EM reconstructions. Theses models are then used as beginning and end points for simulations of large-scale conformational changes. Our strategy has been highly successful in the case of the accommodation conformational change during tRNA selection in bacteria. Here, we correctly predicted the universally conserved accommodation corridor, which has been verified in several independent experimental studies. We have also recently identified the hybrid corridor, responsible for tRNA hybrid state formation during translocation. Our latest addition to our pipeline is the incorporation of SHAPE probing data describing the mobility of the RNA backbone in solution. We have developed a novel algorithm to generate molecular dynamics simulations highly consistent with SHAPE probing data. We have applied these techniques to eukaryotic ribosomes to investigate their dynamics and conformational changes.

213-Plat
Resolving the Mechanisms of Bacterial Resistance to Macrolide Antibiotics
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Macrolides are a class of commonly used antibiotics that target the bacterial ribosome and prevent protein synthesis in the affected cells. Experimental studies have shown that macrolides bind to the ribosome in the protein exit tunnel (PET), through which the nascent peptide elongates during protein synthesis. Since the peptide cannot pass the macrolide in PET, it dissociates from the ribosome before its synthesis is finished. Unfortunately, due to extensive use of macrolides, bacterial resistance to them has become a growing concern. This resistance has been attributed to methylation or mutation of specific rRNA residues in the ribosome. However, how these changes in rRNA induce macrolide resistance on a molecular level is still unclear. Therefore, we investigated the molecular mechanisms of macrolide resistance using atomistic molecular dynamics simulations. Two commonly used macrolides, erythromycin and azithromycin were studied. We have developed CHARMM36 compatible force field parameters for these macrolides using the Force Field Toolkit in VMD and we simulated them in wild-type, mutated and methylated ribosomes of E. coli. The differences in the interactions of macrolides with wild-type and resistant ribosomes elucidate the molecular origins of the evolved resistance.

Platform: Assemblies and Aggregates

214-Plat
Kinetics of Metal Amyloid-Beta Binding and Efficacy of Ligands Targeting Metal Amyloid-Beta Interactions
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Approaches that prevent metal-Aβ interactions, inhibiting the formation of toxic Aβ oligomers as well as restoring metal homeostasis may have potential as new disease modifying strategies for treating Alzheimer's disease (AD). Significant advances have been made recently in the development of "metal protein attenuating compound" (MPAC) for AD, yet the molecular mechanisms of the efficacy of these compounds are not well understood.
Here we investigate the reaction kinetics of copper binding to Aβ and the sequestering of copper from Aβ by ligands using nanomolar concentrations of Aβ under pseudo-first-order conditions, allowing for the determination of the rate constants, binding affinity and reaction mechanisms. The binding of Aβ to the first copper ion is near diffusion limited at physiological pH, with a small activation barrier. The MPAC drug candidates we tested react with the Aβ-Cu complex significantly faster than the common copper chelator EDTA, providing kinetic evidence for the effectiveness of MPACs. The on/off rates of metal-Aβ binding may have important implications for determining the roles of Aβ in modulating synaptic plasticity and memory. A model that unifies the role of metal and membrane-binding properties of the Aβ peptide in neurotransmission is proposed.

215-Plat
Amyloids. How to Study Them with Two-Dimensional Correlation Spectroscopy
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Human and Bovine Serum Albumins as well as Lysozyme are known proteins that can form amyloid fibrils under destabilizing conditions. The use of well-known proteins that have an easily-controlled aggregation process, and the comparison of these processes between similar proteins from different species, would be helpful in elucidating the aggregation process implicated in many degenerative diseases such as Alzheimer's, Parkinson's or Type II diabetes.
Infrared spectroscopy is a technique suitable to follow amyloid formation because is not dependent on the size of the molecule or in the turbidity of the suspension.
We are trying to extract more of the information enclosed in the infrared amide I band by improving the method of two-dimensional correlational spectroscopy. In our approach, we "slice" the spectra in temporary differences of 10 minutes, what we call 2DCOS moving lapse spectroscopy and then we represent the changes in cross-relation intensity or position with time. The results point to a better way of looking at the time-course events that drive a protein into a proto- and then fibril.

216-Plat
Surface-Catalyzed Nucleation of Amyloidogenic Peptides by Peptide-Specific Templates
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Many soluble proteins self-assemble into fibrillar, β-sheet rich, stable amyloid aggregates, many of which are implicated in diseases. Peptide segments within these proteins form the core amyloidogenic region that drives fibrillation of the protein, and it has been shown that these segments, alone, form fibrillar structures. Amyloid fibers are nucleated via a fiber-dependent pathway in which additional protein adds onto the ends and extends existing fibers. However, it is hypothesized that the walls of existing fibers can also nucleate amyloid fibers in a peptide-specific manner using the solvent-exposed side chains as a template. Using a naturally occurring protein that presents side chains of beta sheet structures but no fiber ends, we have engineered templates specific to amyloidogenic peptides to test this hypothesis. We have found that introduction of these templates drastically alter peptide fiber formation kinetics. These findings suggest that amino acid side chains on the surface of amyloid fibers, and not just fiber ends, play an important role in the formation of amyloid fibers.

217-Plat
Formation of Dynamic Soluble Surfactant-Induced Amyloid Beta Peptide Aggregation Intermediates
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The 40-42 residue Amyloid β (Aβ) peptide forms β-structured oligomers on-pathway to amyloid fibril formation and are linked to neuronal damage characteristic for Alzheimer's disease. Surfactants such as SDS induce relatively stable β-structured Aβ co-aggregates and may be considered as a model system for lipids. We have characterized different intermediate aggregation states appearing during the Aβ aggregation process, as well as the kinetics of their formation and dynamic exchange between free and bound peptide. A broad range of biophysical techniques were used, including small angle X-ray scattering (SAXS) and NMR spectroscopy, particularly 15N-CPMG relaxation dispersion experiments.
Aβ shows a three-state secondary structure transition depending on surfactant concentration, from random coil-like, via β-structure to α-helix at high surfactant concentration. Structural information on the β-structured co-aggregates was obtained by SAXS experiments that at the beginning showed a large fraction of globular co-aggregates (diameter ~ 75 Å). This fraction gradually vanished on a min to hr timescale and elongated co-aggregated fibrils were formed (diameter ~ 60 Å and length > 350 Å), in line with transmission electron microscopy images. A fast dynamic exchange process (kex ~ 1100 s-1) between free and co-aggregate bound peptide takes place, as monitored by NMR relaxation dispersion experiments.
This study of the surfactant-induced Aβ aggregation may serve as a model for aggregation of Aβ alone or in the presence of lipids.
Reference:
1. Abelein, A. et al., J. Biol. Chem. (2013), 288, 23518-23528.